

Dredging Research

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Development of assays for genotoxicity testing in dredged sediments

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Federal and state regulatory requirements for detection of contaminants in dredged material intended for open water disposal continue to become more stringent. Testing for contaminants by type and by level can be time-consuming and expensive. In 1990, the need for rapid and accurate testing resulted in U.S. Army Corps of Engineers research under the Long-Term Effects of Dredging Operations (LEDO) Program to develop dredged sediment genotoxicity bioassays for application in regulated disposal operations. In this context, the term "genotoxicity" (Fig. 1) broadly encompasses all carcinogenic, mutagenic, or teratogenic effects of chemically contaminated sediments in aquatic biota, whether mediated through genetic or epigenetic mechanisms. Generally, regulatory evaluation of contaminated sediments for dredging and disposal is based on acute toxicity and bioaccumulation. Existing bioassay methods were not considered appropriate for detection of genotoxic effects on aquatic organisms of dredged material proposed for open-water disposal. In order to formally evaluate existing

bioassays for use as screening tools for genotoxicity in sediments, a workshop was held in March 1990 to gain guidance from recognized authorities on this matter. Participants agreed there were three critical categories of biomarkers: integrators of genotoxic effects, indicators of genotoxicity, and markers of exposure.

Integrators of genotoxic effects

Integrators of genotoxicity include:

- ↳ Tumor or cancer formation studies.
- ↳ Teratogenic studies.

These assays are the most definitive for recognizing genotoxicity, since they integrate all factors that act epigenetically or directly on the genetic material. The panel determined that for this category, a fish tumor model and/or an invertebrate cytogenetic or teratogenic model would best fulfill the needs of the Corps. However, tumor models typically require a large number of organisms and at least three months of exposure in fish. Due to the need of USACE for a rapid screening assay, research concentrated on

developing indicators of genotoxicity and markers of exposure.

Indicators of genotoxicity

Indicators of genotoxicity include assays such as:

- ↳ Mutagenicity tests in bacterial or cell cultures.
- ↳ Cytogenetic tests (for DNA damage discernible at the cellular level) such as chromosomal aberrations.
- ↳ Tests for damage to DNA, such as DNA strand breaks and unscheduled DNA synthesis.

While these tests allow rapid sample screening, they typically rely on exposures of bacteria or cell cultures to sediment extracts rather than intact sediments, and therefore do not take bioavailability of the genotoxicants into consideration.

Biomarkers of exposure

Biomarkers of exposure include assays for:

- ↳ Bile metabolites.
- ↳ DNA adducts.
- ↳ Alterations in protein or enzyme levels.



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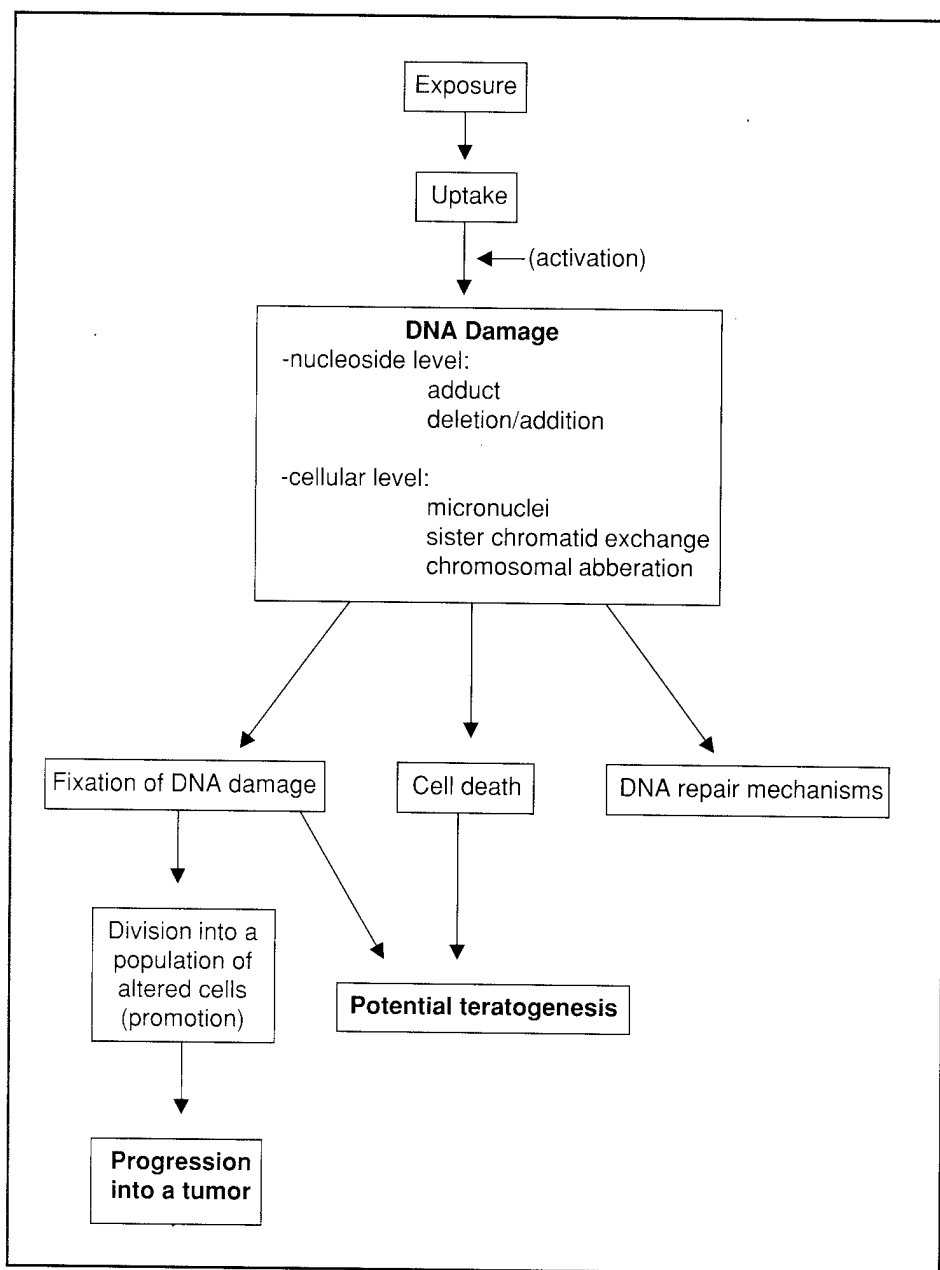


Figure 1. Brief summary of genotoxicity. Genetic mechanisms act through directly damaging DNA, while epigenetic mechanisms can act by increasing fixation of DNA damage, promotion of the altered cells, progression of the altered cells into a tumor, or inhibiting DNA repair. Teratogenesis can result from mutation or death of cells in early development.

These assays indicate that an organism has been exposed to potential genotoxicants, and typically require the exposure of whole organisms directly to the sediments. Although they take bioavailability into account, positive results in these endpoints do not necessarily mean that the organisms will develop tumors.

Research and development

Background. Ideally, assays would be developed for all three categories and could be applied to the fourth tier (chronic sublethal tests/risk assessment) in the standard tiered-testing approach for evaluating dredged material. However, due to the need of USACE for a rapid and cheap assay,

research concentrated on developing indicators of genotoxicity and markers of exposure which are easily adapted to a screening approach (see Fig. 2). Selection of appropriate biomarkers of exposure and indicators of genotoxicity depends not only on correlating genotoxicant exposure to a response in the biomarkers, but correlating the biomarkers to the development of tumors as well.

In vitro Assays. Two cell-based assays (101L and H4IIE) were tested for their ability to detect polycyclic aromatic hydrocarbons (PAHs), coplanar polychlorinated biphenyls (PCBs), dibenzodioxins (PCDDs) and dibenzofurans (PCDFs). These compounds are among the most commonly encountered contaminants in dredged sediments, and include carcinogens, procarcinogens, and promoters of carcinogenicity. Exposure to these compounds results in increased production (called induction) of cytochrome P4501A1, an enzyme which can be measured as a general indicator of genotoxic potential. Both assays have limits of detection for benzo-[a]-pyrene equivalents (BaP-EQ) and 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQ) of 5-10 ng/g sediment. Recently, both assays were modified to a 96-well format. The P450RGS cell-based assay results in a 30-percent time and expendable material cost savings compared to the H4IIE assay.

While these cell-based assays indicate the presence of some classes of genotoxic substances in sediment extracts (PCBs, PAHs, PCDDs, PCDFs), they do not screen for other types of mutagenic substances (direct acting mutagens, nitroaromatic compounds, etc.). The standard Ames and the Mutatox tests are both capable of detecting potential mutagens in sediment extracts by dosing bacterial cultures and quantifying the resulting mutants. The major disadvantage of these tests is that they can be applied only to extracts of sediments, and thus do not address bioavailability.

	Endpoint measured	Ease of use	Time to results	Cost	Relevance	Comments
Indicators of Genotoxicity						
Ames assay ¹	Bacterial mutation (revertants)	Moderate	Moderate	Moderate	High	Uses sediment extracts
Mutatox assay ¹		Easy	Moderate	High to moderate	High	Uses sediment extracts High set-up costs
Modified SOS chromotest		Easy	Moderate	Moderate	High	Uses whole sediment
Biomarkers of Genotoxicity						
H4IIE cell line ²	Enzyme induction	Moderate	Moderate	Moderate	Moderate	Uses sediment extracts Responds to select group of carcinogens
P450RGS cell line ²		Easy	Fast	Low	Moderate	Uses sediment extracts Responds to select group of carcinogens
Antioxidant enzyme assays ³ (in brown bullhead)		Moderate	Moderate	Low	Moderate	Difficult to interpret due to complex interactions in bullhead
EROD assay ³ (in brown bullhead)		Easy	Fast	Low	Moderate	Difficult to interpret due to adaptation in bullhead
ssDNA assay ³ (in brown bullhead)	DNA damage	Moderate	Moderate	Low	High	Not responsive to short-term lab exposures, may be difficult to interpret due to adaptation in bullhead
Transgenic worm assay ⁴		Difficult	Slow	High to moderate	High	Expensive to develop, costs low once standardized

¹ For further information on comparison of Ames and Mutatox, see Technical Report D-95-1.

² For further information on comparison of H4IIE and P450RGS cell lines, see Technical Note DOER-C8.

³ For further information on biomarkers of genotoxicity in brown bullhead, see Technical Note EEDP-04-31.

⁴ For further information on the transgenic worm assay, see Technical Note EEDP-01-43.

Figure 2. Genotoxicity assay attributes

The latest bacterial mutagenicity assay tested at ERDC is the modified SOS Chromotest, which can be applied directly to sediments. The standard SOS Chromotest, which uses sediment extracts, was compared to the modified SOS Chromotest applied to the whole sediments. Although toxicity of the whole sediments to the bacteria complicated data analysis, there was a striking difference in mutagenicity between sediments and their extracts. Eleven of twelve sediment extracts were shown to be genotoxic, compared to none for the whole sediments, indicating that while mutagens were present in the sediments, they were not bioavailable.

These *in vitro* tests indicate that while genotoxicants are common in sediments, they are not necessarily

bioavailable. This emphasizes the importance of developing an *in vivo*, whole-sediment test for genotoxicity to minimize false positive results.

***In vivo* Assays.** In order to correlate biomarker responses to tumor development, the brown bullhead catfish was selected as a model fish (Fig. 3). This



Figure 3. The brown bullhead catfish, *Ameiurus nebulosus*

fish lives in close association with sediment and is highly susceptible to tumor development. A wide array of enzyme assays with the potential of being biomarkers of exposure to genotoxics were developed and most adapted to the 96-well microtiter plate format to increase the speed of the assays (Fig. 4). The ability of the biomarkers to predict potential tumor formation was determined by measuring the various biomarkers in fish from a relatively pristine site and comparing them to those of fish from a highly contaminated site where a high rate of tumor development has been documented. The response of the biomarkers was also compared against the ssDNA assay, an indicator of genotoxicity.

Although initial results indicated several of the biomarkers correlated to exposure (the antioxidant enzymes superoxide dismutase and catalase, and total glutathione), two assays indicated that fish from the control site were exposed to genotoxics (EROD, ssDNA assay). These unexpected findings were thought to result from the combination of adaptive response in fish from the contaminated site and low-level contamination being present at the reference site. In an attempt to clarify the issue, a series of laboratory and field exposures were conducted. Final results indicated that the population native to the contaminated site may have adapted to the presence of chemical contaminants by lowering constitutive levels of EROD, the enzyme responsible for activating PAHs to the reactive metabolites that ultimately lead to DNA damage. A follow-up investigation was conducted in order to confirm the biomarker responses observed in the initial field study, and results failed to confirm previously observed trends in superoxide dismutase, catalase, and total glutathione. The initial field study findings may have been influenced by transitory, low-level PAH contamination at the control site due to low water flow. EROD levels at the

Assay name	Description
GSH	An assay which determines the total glutathione content, a natural antioxidant
ssDNA	An assay which can detect single strand breaks in DNA, a measure of damage to DNA (not a 96-well format assay)
EROD	An assay for Ethoxyresorufin-O-deethylase, Phase I detoxification enzyme
SOD	An assay for superoxide dismutase, an antioxidant enzyme
CAT	An assay for catalase, an antioxidant enzyme
GR	An assay for glutathione reductase, an antioxidant enzyme
GPX	An assay for glutathione peroxidase, an antioxidant enzyme
GST	An assay for glutathione S-transferases, a Phase II detoxification enzyme

Figure 4. Biomarkers in livers of brown bullhead. All assays are adapted to the 96-well format unless noted

control site in 1999 were lower than that of control site fish from the 1996 collection, indicating a decrease in PAHs over time. Correspondingly, the ssDNA levels of control site fish no longer indicated DNA damage at the control site. This response supports the use of the ssDNA break assay as a good marker for low-level contamination in populations not chronically exposed to high levels of PAHs.

Overall, the bullhead studies indicated that while both ssDNA break assay and EROD assay are both potentially useful for detecting genotoxicity in sediments, the use of native populations for biomonitoring purposes can be misleading due to adaptive responses in fish living in chronically contaminated sites.

Future studies. Our *in vitro* studies with bacterial and cell cultures indicated that testing sediment extracts could have only limited utility, since mutagens were likely to be present in practically all sediments. Unless the question of bioavailability is addressed in a test of sediment genotoxicity, very little information can be obtained about actual risks. A model with a practical method for identifying and quantifying mutations that occurred in the animals' DNA due to contact with the bioavailable mutagens in sediments would ideally address this issue. For this reason the development of a trans-

genic organism, designed to detect bioavailable mutagenic compounds in sediments, was proposed. A transgenic animal is one that has had foreign DNA (a transgene) inserted into its genome. The approach taken is one that has been used successfully in fish, mice, and rats. A transgene is inserted into the gametes, which develop into an adult organism that contains the transgene in all cells. Exposure of the organisms to genotoxics results in damage to DNA, including the transgene, which can then be recovered from the organism and mutated transgenes amplified and detected via a bacterial host. Two species of polychaete worms were selected as appropriate organisms for sediment testing, as both burrow in the sediment and process it for food (Fig. 5). Currently, the gene has been successfully inserted into *Armandia* gametes, and this first transgenic generation has been successfully settled from the planktonic larval stage. When mature, the adults will be used to breed a second generation of transgenic worms as well as to begin characterizing their sensitivity and selectivity to known mutagens and carcinogens. Once the transgenic worm line is characterized, it can be used for *in vivo* testing of whole sediments.

Development and characterization of the transgenic worm is a major undertaking, but it provides many

advantages. One advantage is that the type of mutation can be determined with the transgenic assay, providing information critical to interpretation of the potential effects of the mutation. For example, a mutation that results in the inability of a gene to encode an essential enzyme or other protein can lead to the death of the mutated cell. While this may not lead to the formation of a tumor, it could be disastrous in a developing embryo where the death of a single cell at a critical time and place may result in severe birth defects (teratogenesis). On the other hand, large base deletions and point mutations are known to result in activation of oncogenes, which are genes that when activated contribute to the emergence of malignant tumors. Thus, the ability to determine what type of DNA damage is occurring greatly improves the ability to predict the potential effects. Another advantage of the transgenic assay is that recent research has shown that low levels of damage to DNA results in decreased growth, reproduction and survival; thus, results can be interpretable in terms of ecological risk assessment. Finally, an extremely important aspect is that the assay possesses the ability for the relatively rapid and inexpensive assessment of the bioavailable fraction of genotoxins in whole sediments.

For additional information, contact Dr. Laura S. Inouye, (601-634-2910, inouye1@wes.army.mil) or Dr. Victor A. McFarland (601-634-3721, mcfarlv@wes.army.mil). Technical notes on many of the topics discussed are available at www.wes.army.mil/el/dots/pubs.html; for comparison of Ames and Mutatox see TR D-95-1; for information on the H4IIE and 101L cell lines, see TN DOER-C1 and TN DOER-C8; for biomarkers of genotoxicity in brown bullhead see TN EEDP-04-31; and for the transgenic worm assay, see TN EEDP-01-43.

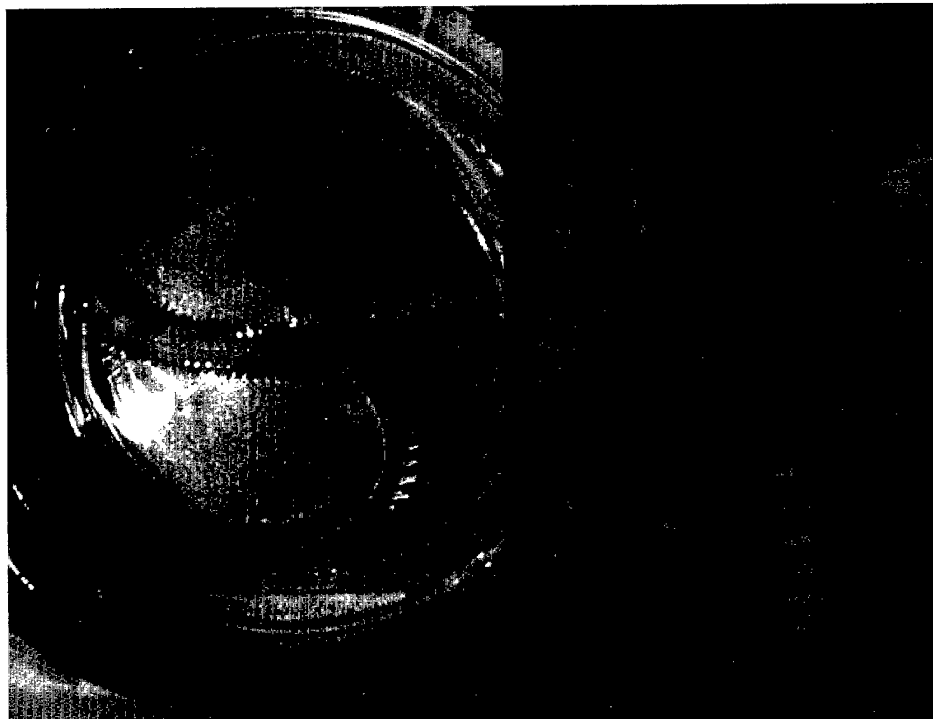


Figure 5. The polychaete worms being used for the transgenic project. *Neanthes succinea* on the left, *Armandia brevis* on the right (photo of *Armandia* courtesy of Casey Rice, NOAA/NMFS, Seattle, WA).

Articles for Dredging Research requested:

Dredging Research is an information exchange bulletin for publication of ERDC-generated dredging research results. Included are articles about applied research projects. The bulletin serves all audiences and is accessible on the World Wide Web in addition to a paper circulation of 2,800.

Articles from non-ERDC authors are solicited for publication, especially if the work described is tied to the use of ERDC-generated research results. Research articles that complement ERDC research or cover wide field applications are also accepted for consideration. Manuscripts should use a nontechnical writing style and should include suggestions for visuals and an author point of contact. Point of contact is Elke Briuer, APR, at briuer@wes.army.mil.

Center for Contaminated Sediments launches new Website



Center for Contaminated Sediments

U.S. Army Corps of Engineers | Engineer Research and Development Center
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Mission

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Technology Transfer

Related Sites

Local related links:

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- DOTS
- Environmental Chemistry
- Hazardous Waste

Michael R. Palermo, Ph.D. Director

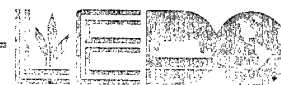
The U.S. Army Corps of Engineers Center for Contaminated Sediments (CCS), consolidates research expertise to deal with the problem of contaminated sediments. The Center coordinates and facilitates contaminated sediment activities among Corps organizations, the Department of Defense, other federal and state agencies, academia, and the private sector.

Research and development activities support the Corps of Engineers navigation mission as well as work related to military cleanup activities; the Environmental Protection Agency's Superfund and Assessment and Remediation of Contaminated Sediments Programs; and the National Oceanic and Atmospheric Administration's Natural Resource Trustee Program.

visit at <http://www.wes.army.mil/el/dots/ccs/ccs.html>

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Volatile Contaminant Loss from Dredged Material



The loss of volatile contaminants from sediments and dredged materials is an increasingly recognized environmental problem. Disposal and storage operations associated with dredged material placement in confined disposal facilities (CDFs) can result in volatile compound emissions. Research was conducted by ERDC scientists to develop predictive exposure assessment methodologies describing the loss of volatile organic compounds (VOCs) from dredged material management sites. Such methodologies will allow sites to be managed for VOC losses. Initial research efforts focused on the development of a laboratory apparatus and procedures to obtain experimental

data on the emission of VOCs from exposed sediment. Data generated from measurements of emissions from laboratory-spiked and field contaminated sediments have been used to validate proposed mathematical models for estimating volatile emissions from contaminated sediments. A controlled field simulation experiment was conducted with a field contaminated sediment to verify laboratory experimental results. Measured contaminant emissions from the laboratory and field simulation experiments agreed well with model predictions. The model is currently being modified for incorporation into the Automated Dredging and Disposal Alternative Modeling System

(ADDAMS) suite of models (available from the ERDC Environmental Laboratory Website (<http://www.wes.army.mil/el/dots/models.html>)). Once available, it can be used to generate initial screening data for maximum contaminant fluxes from freshly deposited dredged material.

Additional information is available from Ms. Cindy Price (pricec@wes.army.mil), a scientist who is the principal investigator for the project. Look for an expanded article on this subject in the June 2000 issue of *Dredging Research*.

Dredging Calendar

January 2000

January 11-13 - Dredged Material Assessment & Management Seminar, sponsored by USACE and USEPA, in San Diego, CA.
POC: skinneb@wes.army.mil or
www.wes.army.mil/ell/dots/training/register.html

January 30-February 3 - 54th Annual Meeting of National Association of Conservation Districts, in Colorado Springs, CO.
POC: reschke@macdnet.org

February 2000

February 7-10 - Tools for Urban Water Resource Management and Protection; A National Conference, sponsored by EPA et al., in Glencoe, IL.
POC: bkirsch@chicagobotanic.org

February 13-17 - 10th International Zebra Mussel and Aquatic Nuisance Species Conference, in Toronto, Ontario,
POC: Elizabeth Muchkle-Jeffs, (800) 868-8776 or
profedge@renc.igs.net

February 23-24 - Great Lakes & Ohio River Division Water Quality Workshop, in Nashville, TN.
POC: joe.e.svirbely@usace.army.mil

February 23-26 - Wolves: A Global Symposium, hosted by the International Wolf Center and the University of Minnesota-Duluth, in Duluth, MN.
POC: merickso@d.umn.edu

March 2000

March 7-8 - Water Quality Conference 2000, sponsored by Iowa State University, in Ames, IA.
POC: http://extension.agron.iastate.edu/aged/water_quality/wqconf.html

March 15 - Great Lakes Congressional Breakfast, sponsored by the Great Lakes Commission and Great Lakes Congressional Task Force, in Washington DC.
POC: mdonahue@glc.org

Dredging Products

The following technical notes were published for the DOER program during 1999. The technical notes can be found in .pdf format at <http://www.wes.army.mil/ell/dots/doer/technote.html>

DOER-C2	Dredged Material Characterization Tests for Beneficial Use Suitability (May 1999)
DOER-C3	Evaluation of Dredged Material for Phytoreclamation Suitability (May 1999)
DOER-C4	Screening Tests for Assessing the Bioreclamation of Dredged Material (May 1999)
DOER-C5	Bioremediation of PAH-Contaminated Dredged Material at the Jones Island CDF: Materials, Equipment, and Initial Operations (September 1999)
DOER-C6	Manufactured Soil Screening Test (May 1999)
DOER-C7	Case Studies: Characterization Tests to Determine Dredged Material Suitability for Beneficial Uses (July 1999)
DOER-C8	Comparison of Two Cell-Based Assays for Screening Dioxin and Dioxin-Like Compounds in Sediments (July 1999)
DOER-C9	Phytoreclamation of Dredged Material: A Working Group Summary (November 1999)
DOER-E6	Estimating Dredging Sediment Resuspension Sources (March 1999)
DOER-N4	MODEL: Sediment Grain-Size Depth of Residence (May 1999)

The following technical notes were published for the EEDP program during 1999. The technical notes can be found in .pdf format at <http://www.wes.army.mil/ell/dots/eedptn.html>

EEDP-01-43	Development of a Transgenic Model to Assess Bioavailable Genotoxicity in Sediments (April 1999)
EEDP-02-26	Volatile Losses from Aged Field Sediments (January 1999)
EEDP-02-27	Application of SLRP to Pearl Harbor Dredged Material (August 1999)
EEDP-04-30	Interpreting Bioaccumulation Data with the Environmental Residue-Effects Database (January 1999)
EEDP-04-31	Biomarkers of Oxidative Stress and Genotoxicity in Livers of Field-Collected Brown Bullhead (September 1999)
EEDP-04-32	Analysis of Uncertainty in TBP Estimation of PAH Bioaccumulation Potential in Sediments (June 1999)
EEDP-06-20	Documentation of the Hydrologic Evaluation of Leachate Production and Quality (HELPQ) Module (January 1999)
EEDP-06-21	ADDAMS Application: Hydraulic Evaluation of Leachate Production and Quality (HELPQ) Module in CDFs (January 1999)



**US Army Corps
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***Organizational news: ERDC consolidates
R&D functions***

On Oct. 1, 1998, the U.S. Army Corps of Engineers created the U.S. Army Engineer Research and Development Center (ERDC) by consolidating the administrative functions of the four existing Corps R&D organizations (Cold Regions Research and Engineering Laboratory; Construction Engineering Research Laboratory; Topographic Engineering Center; and the Coastal and Hydraulics, Environmental, Geotechnical, Information Technology, and Structures Laboratories of the Waterways Experiment Station). Effective Oct. 1, 1999, the research and development functions were consolidated under ERDC. Visit the ERDC Website at <http://www.erdcl.usace.army.mil> for updates and current information.

Dredging Research

This bulletin is published in accordance with AR 25-30 as an information dissemination function of the Environmental Laboratory of the U.S. Army Engineer Research and Development Center. The publication is part of the technology transfer mission of the Dredging Operations Technical Support (DOTS) Program and includes information about various dredging research areas. Special emphasis will be placed on articles relating to application of research results or technology to specific project needs. The contents of this bulletin are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or the approval of the use of such commercial products. Contributions are solicited from all sources and will be considered for publication. Editor is Elke Briuer, APR, briuer@wes.army.mil. Mail correspondence to the Environmental Laboratory, ATTN: DOTS, *Dredging Research*, U.S. Army Engineer Research and Development Center, Waterways Experiment Station (CEERD-EP-D), 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, or call (601) 634-2349. Internet address: www.wes.army.mil/el/dots/drieb.html.

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